

(new claim 28, continued)

B2  
Entitled  
host cell of claim 24 and culturing said host cell under conditions permitting expression of the protein.

91  
--29 (new). A procoagulant protein having a peptide sequence substantially the same as that of human Factor VIII:C but lacking a peptide region ~~within a region~~ selected from the group consisting of:

- (a) the region between Pro-1000 and Asp-1582;
- (b) the region between Thr-778 and Pro-1659; and,
- (c) the region between Thr-778 and Glu-1694.

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--30 (new). A pharmaceutical preparation for the treatment of Hemophilia A comprising a sterile preparation containing an effective amount of a protein of claim 29, in admixture with a pharmaceutically accepted carrier.

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--31 (new). A method for treating Hemophilia A comprising administering to a patient a pharmaceutical preparation of claim 30.--

#### REMARKS

Applicant thanks the Examiner for her thorough review of this application. The application has now been amended to recite its status as a continuation in part of the prior co-owned (at all times) parent application.

The original claims, claims 1-19 have been replaced with new claims 20-31 which are intended to more clearly and simply define the subject matter sought to be claimed, and to do so without reliance on Table 1 of the specification. Support for the new claims should be evident e.g. by comparison to the original claims. No new matter is believed introduced by this amendment.

42

JJ Toole - Response  
USSN 010,085  
page -4-

Since the peptide sequence for human Factor VIII:C (FVIII) as depicted in Table 1 is known in the art and was so known as of the instant filing date, its specific inclusion as a figure or reference within the claims is not believed to be necessary.

Turning to the issues raised by the Office Action, the claims as amended above are believed to distinguish over products of nature as well as over any products disclosed in the cited art concerning recombinant FVIII, i.e., Wood et al, Vehar et al, or Toole et al. The amended claims all relate to FVIII variants, products containing them, and DNA encoding them, wherein a number of amino acids otherwise found in the peptide sequence of FVIII (as a natural or recombinant product) are missing. Support for that limitation is found in the specification e.g. on page 2, lines 28-30 which states that the region between the FVIII heavy and light chains "may comprise a continuous but shorter sequence selected from the region Ser-760 through Arg-1708"; on page 12, lines 1-3, which discloses that the proteins of this invention lack a substantial amino acid segment of human FVIII; on page 13, first new paragraph, which discloses that such deletion variants may be produced by expressing a DNA prepared by cutting a full length FVIII DNA to remove a portion of the DNA sequence encoding amino acids 760 to 1708; and from the examples of deletion variants provided in the specification (see e.g. Table 2 on page 11).

As to the remaining §112 issue, the relevant claims have been limited to mammalian host cells, thus rendering moot the ground for rejection.

Applicant's comments with respect to the other rejections based on cited references are as follows.

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1. Wood et al is not believed to disclose or suggest in any way the possibility that deletion variants of FVIII could be produced or if produced would be active procoagulant proteins. The pending claims as amended above are limited to deletion variants of FVIII and thus are believed to be clearly distinguished.

2. Based on Wood et al, Vehar et al and Toole et al, it was suggested in the Office Action that the art taught (a) that B domain removal activates FVIII, (b) that cleavage sites permitting removal of the B domain were reported and (c) that deletion of that domain as in the present invention would be an obvious expedient for expressing and isolating the protein more easily since the protein produced would be smaller and not contain the heavily glycosylated portion of the protein. According to that analysis, it would have been obvious to determine which portions of FVIII could be deleted without loss of procoagulant activity simply by cloning successively larger internal deletions, absent unexpected results. Furthermore, it was considered expected that much of the B domain could be deleted since Wood et al reportedly taught that FVIII fragments as small as 90 and 80 kD comprise procoagulant activity.

Applicant does not share that view, and is optimistic that the Examiner will reconsider her initial opinion in view of the following discussion.

(a) Removal of the B domain does not activate FVIII

As shown in the subject application (p. 21), the DGR-2 and LA-2 variants are thrombin (IIa) activatable to about the same extent as wildtype FVIII, i.e. approximately 20-30-fold in the assay employed. If removal of the B domain activates FVIII, then the deletion variants would already be activated inherently, and would not be susceptible to further thrombin-induced activation,

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at least not on the order of wildtype FVIII. In view of the data presented within the application itself, it is clear that removal of the B domain does not activate FVIII and that conclusions based on that erroneous assumption would also be wanting. Indeed, ascertaining that removal of the B domain does not activate FVIII forms part of applicant's contribution to the art. See also, Toole et al, 1986, PNAS 83:5939-5942, copy enclosed with IDS.

Furthermore, FVIII fragments as small as 90 and 80 kD are simply not individually active. See Ref. AA (Chiron), especially page 7, lines 8-10; and Ref. AAA (Eaton et al), especially page 3287.

(b) Reports of cleavage sites defining the  
B domain do not suggest B domain deletions

Wood et al disclose that plasma-derived FVIII usually "consist of a set of fragments between  $M_r$  90,000 and 210,000 with very similar tryptic maps and another, with  $M_r$  of 80,000 with distinctive map. ... The peak of factor VIII activity found upon thrombin incubation correlates with the enhanced presence of the  $M_r$  90,000 and a 70,000 fragment."

Significantly, Wood et al are not believed to disclose that only the 70 and 90 kD fragments are required for activity, but merely that enhanced presence of those two fragments among other fragments present in a mixture correlated with peak activity in their experiments. Thus, based on Wood et al it is not valid to conclude that the B domain or other components are not also required for activity.

Furthermore, neither Wood et al nor Vehar et al disclose or suggest that FVIII can be produced without the B domain or that if it could that the FVIII would be active. Indeed, in the case of insulin, another protein which contains an internal region which

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is subsequently deleted, the internal region is believed essential in the normal biosynthetic folding of the nascent polypeptide from which the internal region is only subsequently removed. As to FVIII, the Examiner herself correctly noted on page 3 of the Office Action that FVIII requires specific intracellular processing including glycosylation and specific cleavage events. Furthermore, the complex FVIII protein must be properly post-translationally modified in other respects and must be properly folded. Vehar et al specifically recognize that the B domain may indeed play a role in intracellular processing of FVIII:

The portion of the protein [B domain] immediately prior to the  $M_r$  80,000 protein may thus be a disulphide linker region which holds the  $M_r$  210,000 and 80,000 (or larger precursors) in the reducible 330,000 form. (page 341, right column, 1st new sentence below Fig. 6)

That the internal region of FVIII is not necessary for proper folding and assembly for procoagulant activity is another of applicant's key contributions to the art. Prior to applicant's teaching, there was no reason to suspect that to be true. Indeed, Vehar et al provide a motivation for disbelief, or at least surprise, at that finding.

Thus while Wood et al and Vehar et al do teach a domain structure for FVIII they do not disclose or suggest in any way the desirability or possibility of deleting portions of the FVIII protein, producing such proteins by recombinant means or pharmaceutical preparations containing such deletion variants.

(c) B domain deletion is not an obvious production expedient

It is true that low production yields for recombinant FVIII production have been reported subsequent to applicant's filing date. However, to applicant's knowledge, production problems for recombinant FVIII had not been reported in the prior art and,

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furthermore, some of the causes of those low yields have only recently being elucidated, as discussed below. The Office Action speculated that a FVIII deletion variant encoded by a cDNA deleted within the B-domain encoding region would be expected to be more easily expressed and isolated since it would be smaller and not include the heavily glycosylated B domain. However, that speculation appears to be only an attempted ex post facto explanation of applicant's results, rather than an a priori reasonable expectation in view of only the prior art. The failure in the art to recognize the very existence of any actual production problem for recombinant FVIII, and thus the absence of motivation for enhancing expression levels, clearly indicates that the position of the Office Action is only a retrospective conclusion.

Returning to the vantage point of applicant's effective filing date, the art failed to recognize any actual FVIII expression problem, and specifically failed to recognize that the size of FVIII and the B-domain glycosylation led to production problems. Indeed, those factors per se do not appear to contribute to low FVIII yields, especially, as it turns out, not for the reasons presented in the Office Action. FVIII is smaller and contains fewer carbohydrate moieties than vWF, a protein whose high level expression is uneventful. Furthermore, Factor V, a protein of similar size to Factor VIII and also having a heavily glycosylated B domain is also expressed uneventfully. Thus, there would be no reason to believe that the size or glycosylation status of FVIII were causally related to low FVIII production levels. Since those factors would not a priori be suggested to one of ordinary skill in the art as problems, especially given that expression of comparable proteins is not problematic, it would not have been logical to delete a portion of FVIII having a function which remains unknown to this date - absent applicant's contribution.

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Indeed, the role, if any, of the B domain in the biological functioning of FVIII was not known and is still not known. Even after applicant's discovery it was still suggested that the B domain may protect FVIII from proteolysis (see Eaton et al, Ref. AAF, page 8346, third sentence from end) and that the B domain may be important for interaction with vWF (although this is no longer believed true) or may be involved in intracellular processing or storage in the cells that normally produce FVIII in vivo or even that the B domain or proteolytic products derived therefrom) have procoagulant, anticoagulant or vasoactive properties theretofore unknown (see Toole et al, Ref. AAB, page 548, last two paragraphs prior to "Acknowledgements").

Since FVIII is desirable precisely because of its collective set of specific functional properties, it would not have been logical a priori as of applicant's effective filing date for one of ordinary skill in the art to simply delete a portion of the protein having unknown functionality simply because it is there. Furthermore, even if one were to contemplate that illogical exercise, there would be no requisite basis a priori for expecting that desired functional properties of the parent protein would be retained, even if it could be properly expressed.

As it turned out, applicant did find to his surprise that the FVIII deletion variants are active and are expressed at significantly higher levels than wildtype FVIII. That finding is another of applicant's key contributions to the art and is confirmed by other groups who subsequently reproduced applicant's invention [see refs AA-AE of IDS]. Applicant's finding was surprising because the increase in expression level was so substantial and because the reason for the increased expression level was unknown.

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Applicant and the subsequent groups did speculate initially as to the reason for the increased expression level observed for the deletion variants, and it has been suggested that their smaller size and lack of a heavily glycosylated internal region is responsible. However, that reason has been ruled out in view of results obtained with Factor V and vWF. In contrast, later work has shown that FVIII expression is significantly hampered as a result of intra-cellular events giving rise to issues much more subtle than the mere size or heavy glycosylation of the protein. For instance, binding of the nascent FVIII protein to an endoplasmic reticulum (ER) protein referred to as GRP72 or BiP and sequestering of the nascent protein as a complex in the ER followed by eventual degradation reduces observed FVIII expression levels. It has been found that the deletion variants of FVIII bind less well or more transiently to BiP, and therefore do not suffer a hold-up in the ER [See Refs AI and AAH]. However, even had the existence and role of BiP had been known in the prior art, it still would not have obviated FVIII deletion variants, absent some suggestion in the art to make such deletions. Indeed, even until subsequent experiments were conducted, there was no reason to believe that a BiP binding site existed anywhere in the FVIII protein or that such a site existed in the FVIII B domain. Even today there is some controversy in the art as to what factors are responsible for the binding of BiP to a nascent protein [See Refs AAH and ABC].

In view of the lack of suggestion or motive for removing the B-domain or a B-domain-encoding nucleotide region provided by the references or any prior art basis for reasonably believing that such deletion variants would even be capable of production, the combination of Wood et al, Vehar et al and Toole et al is, with all due respect, no more than a retrospective reconstruction of applicant's invention. However, the would-be combination must

49

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49

JJ Toole - Response  
USSN 010,085  
page -11-

fail since none of the references provide a sufficient motivation for making any deletion modification as is disclosed for the first time in the subject application or the desirability or possibility of this invention as claimed.

Furthermore, even if such a suggestion could be found in the references, and applicant finds no such suggestion, there would be no reason to believe that a deleted DNA sequence would even be capable of proper expression and secretion. Indeed, in view of the Vehar et al disclosure of a potential role for the B domain in the intracellular processing of FVIII, one would conclude, if anything, that the B domain should not be deleted since proper processing of FVIII protein may be impeded or prevented. Such prior art only highlights the non-obviousness of this invention.

Finally, and in view of all of the preceding, applicants respectfully submit that the cited references, whether considered individually or in combination, by no means disclose or suggest the desirability, utility or even possibility of this invention.

Applicant also submits herewith an Information Disclosure Statement identifying references of possible interest. Some of the references are prior to applicant's filing date, while others are subsequent. None of the references are believed to have any negative impact on the patentability of the pending claims.

In view of the above, applicants respectfully request that each of the grounds for objection and rejection be reconsidered and withdrawn, and the pending claims be allowed. If further discussion of these or other issues would be helpful, the Examiner is invited to call the undersigned attorney at the number below.

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JJ Toole - Response  
USSN 010,085  
page -12-

No fee is believed due in connection with filing this Response.  
If any fee should be due, authorization is hereby given to charge  
the amount of any such fee to Deposit Account 07-1060.

Respectfully submitted,

*David L. Berstein*

David L. Berstein  
Reg. No. 31,235  
Attorney for Applicant  
(617) 876-1170 ext. 394

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*David L. Berstein*

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